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THE EFFECTS OF EPIDERMAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR ON EPITHELIALIZATION OF MESHED SKIN GRAFT INTERSTICES

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INTRODUCTION

Epithelialization is one aspect in the process of wound repair. Others include contraction, collagen degradation and synthesis, fibroplasia, angiogenesis, and inflammation. These processes involve different cells, secreting or reacting to a myriad of signals, in a temporal environment. Humans heal by epithelialization when the wound or injury is small or of partial thickness; that is, when the distance of migration from the wound edges is relatively short, or the follicular structures of the integuement are intact. The remaining epidermal cells become highly proliferative and repopulate the wound surface via migration. Signals to initiate and maintain proliferation can come from more than one cell type, via more than one physiologic message. This is the role of growth factors. They act within the wound in a controlled, temporary fashion. This property separates the process of inflammation from that of cancer - the paracrine action of these cells, as regulated by the intrinsic growth factors, causes great growth potential, but in a limited time-frame (Sporn and Roberts, 1986). Thus, the use of physiologic growth factors in a clinical setting should be

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self-limiting and may have great potential in the manipulation of wound healing, without the fear of uncontrolled repair (Hunt, 1984).

The two growth factors tested in this study were human recombinant epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Both are found in the healing wound (Pessa et al., 1987), have receptors on both fibroblasts and keratinocytes (Shipley et al., 1989), and have been studied for their usefulness in effecting the rate of healing.

EGF was first described by Cohen (Cohen, 1962), while studying the effects of an extract from mouse submaxillary glands. It is a single chain, 53-amino-acid polypeptide (MW = 6045), which is active in controlling the rate of replication of keratinocytes and fibroblasts (Cohen and Elliot, 1963 and Hollenberg and Cuatrecasas, 1973). Many studies have assessed the wound healing properties of EGF, with mixed results. Franklin and Lynch showed improved healing in wounds to rabbit ears (Franklin and Lynch, 1979), both with epithelialization and improved dermal fibroblast organization. Laato also saw increased granulation tissue in a dose-dependent fashion with topical EGF (Laato et al., 1985). Buckley noted the ability of EGF to organize ingrowth of fibroblasts and increase the production of collagen and neovascularization (Buckley et al., 1985). He also made a point that other authors have stated -- the effects of EGF are seen only with sustained exposure of the cells to the growth factor. Other groups have not seen a positive effect in wound healing with EGF. Although Brown showed that EGF in silver sulfadiazine was effective in treating partial thickness wounds in both mice (Brown et al., 1986)

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and clinically (Brown et al., 1989), Thorton saw no such relationship using the same carrier (Thorton et al., 1981). Chvapil saw that the carrier they employed, lanolin cream, had a greater effect on wound healing than EGF (Chapvil et al., 1988).

The increased vascularization and collagen formation in the healing wound could also be enhanced by growth factors other than EGF. Platelet-derived growth factor acts as a potent mitogen for fibroblasts and is also chemotactic for these cells (Pessa et al., 1987). The two heparin-binding growth factors - basic and acidic fibroblast growth factors - are extremely angiogenic in vivo (Folkman and Klagsbrun, 1987), and mitogenic for fibroblasts in vitro (Shipley et al., 1989). Basic FGF is a 146-amino acid polypeptide (MW = 16,000 - 18,500) cleavage product of endothelial cell growth factor. Its relationship to angiogenesis and tumor growth was first described by Folkman (Folkman and Klagsbrun, 1987). The efficacy of bFGF in wound healing has not been explored as extensively as that of EGF. McGee showed bFGF had a positive effect on incisional tensile strength in rats (McGee et al., 1988). For our study, basic fibroblast growth factor (bFGF) was evaluated for its ability to effect epithelialization with and without EGF.

METHODS

Fresh, non-frozen split-thickness skin was obtained from the UCSD Regional Tissue Bank and was meshed with 3 to 1 expansion ratio. Individual grafts were then made by trimming squares with four interstices per side. This allowed for a total of 16 interstices, four of

which would be central, having no contact with the normal mouse skin at the edge of the wound.

Full-thickness skin defects 2 X 2 cm were created on four to eight week old athymic mice (BALB/c - nu/nu) to a depth sparing the panniculus carnosis. Meshed split-thickness human skin grafts as described above were then placed onto the wound, with the bias stretched horizontally. These were secured with 6-0 silk sutures. Test dressings (carrier±growth factors) were put directly onto the graft and XeroformTM petrolatum dressing was then placed. The silk sutures were used to create a stent dressing with these layers. Three Band-AidTM adhesive bandages were then placed to keep wounds isolated from any mouse contact.

Conditions included: 1) Xeroform alone (a standard graft dressing), 2) A 2 X 2 cm piece of GelipermTM alone (carrier control), 3) Geliperm + 10 mcg/cm2 epidermal growth factor (EGF)(Amgen, Incorporated; Thousand Oaks, CA.), 4) Geliperm + 300 ng/cm2 basic fibroblast growth factor (bFGF)(Amgen), and 5) Geliperm + 10 mcg/cm2 EGF + 300 ng/cm2 bFGF. Geliperm is a polyacrylamide gel which can be used for slow release of agents.

Every two days mice were anesthetized, dressings were changed, and wounds were photographed. Closure of interstices was determined at each dressing change in a blinded fashion. Criteria for closure was visual determination of epithelialization of at least three of the four central interstices. Mice were sacrificed on postoperative day eight and grafts were fully excised from each animal. Sections through the four central interstices were obtained

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to determine epithelial thickness, and presence or abscence of human cells.

Samples were obtained for light microscopy to determine epithelial thickness for all closed interstices. Direct immunofluorescence was used to show that human cells populated the closed interstices and not mouse cells migrating from the edges. Frozen sections were made from unfixed samples of the interstice/graft interface. A fluorescein-labelled monoclonal antibody against human HLA-ABC histocompatibility antigens was the positive marker used to make this determination.

RESULTS

The results of this experiment are compiled in Table 1. Data are expressed as percentage of animals "closed" at each postoperative day, and epithelial thickness (micrometers) of the closed central interstices of each graft at time of sacrifice. For the epithelial thickness data, a students' t-test was used for pairwise comparison

TABLE 1. Time to closure and epithelial thickness of wounds treated with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF).

	Percent animals closed Post-op Day					**Epithelial thickness**
CONDITION	**N**	**2**	**4**	**6**	**8**	**mcm**
Xeroform	15	0	13*	47*	87	79 ± 5.2*
Geliperm	22	0	27	55*	86	102± 5.6*
Geli+EGF	22	0	45	82	91	128± 5.4
Geli+bFGF	6	0	17*	0*	33*	N.M.
Geli+bFGF+ EGF	6	0	17*	0*	83	70±10.7*

* $p<0.05$ when compared to EGF treated grafts
N.M. = no measureable epithelial layer

of groups and statistical significance was assumed if p<0.05 (*). Comparison of groups in time to closure was analyzed by the chi-square test. Again, significance was assumed if p< 0.05. Animals were withdrawn from the study for loss of bandage during the study, graft failure or death. There was no correlation of death to any specific dressing condition.

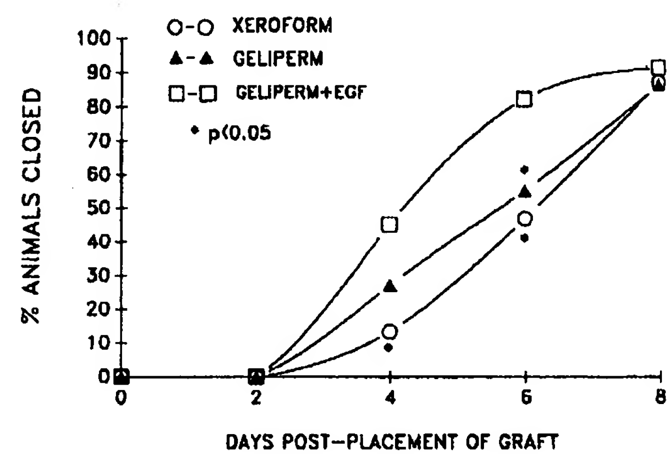

Figure 1. Effect of epidermal growth factor (EGF) on time to epithelialization of meshed skin graft interstices.

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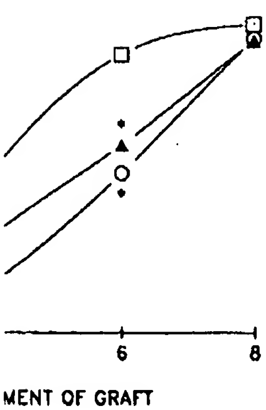

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Time to closure, or epithelialization of at least three of the four central interstices, was signifcantly shorter in the EGF treated grafts (condition 3). This was true when compared to carrier control (condition 2) at day 6, and Xeroform dressing (condition 1) at days 4 and 6 (Figure 1).

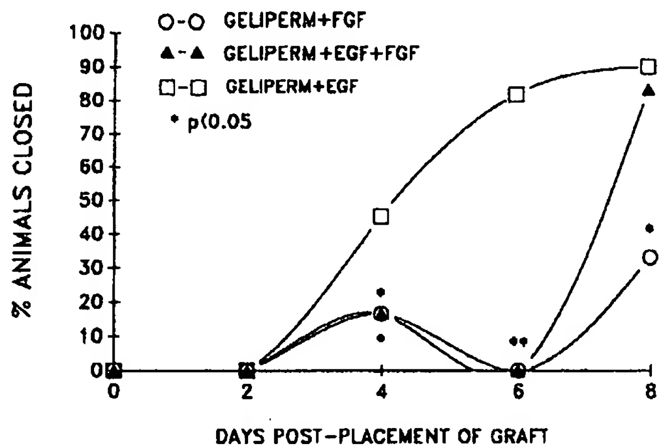

Figure 2. Effects of basic fibroblast growth factor (bFGF) with and without epidermal growth factor (EGF) on time to epithelialization of meshed skin graft interstices when compared to EGF treated grafts alone.

There were no significant differences by day 8. There was no significant difference between the two controls, Geliperm alone (condition 2) and Xeroform (conditon 1). Preliminary data on the bFGF treated grafts (n=6) showed significantly less epithelialization than either EGF or Geliperm alone at all time points after 2 days (Figure 2). From this early data, the presence of bFGF at the tested dose appears to retard epithelialization in this model.

Light microscopy showed that the EGF treated grafts (condition 3) had a significantly thicker epithelial layer when compared to all other conditions (Figure 3). Resultant epithelium with Geliperm + EGF + bFGF was significantly thinner than the carrier control (condition 2), with bFGF alone (condition 4) having no measureable closed interstices.

Random indirect immunofluorescence of the closed interstices showed positive presence of human keratinocytes in all cases. This implies that the epithelial layer which is present has risen from the human graft placed, and not from migration of mouse keratinocytes from the wound edges.

DISCUSSION

The use of agents to enhance wound healing is an expanding area of surgery. Such dissimilar wounds as pressure ulcers, partial thickness burns, open wounds, donor sites, and bone fractures are actively being pursued as areas for the clinical use of polypeptide growth factors. Their ability to increase or stimulate the normal

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inflammation and repair process has not been clearly shown, although ample information implies that their use in treating wounds will be advantagous. The delivery of these growth factors is important in solving this problem. Successful delivery includes: 1) adequate time of exposure of the factor to the wound bed, 2) the product remaining stable, 3) ability to handle fluid from the wound (differs widely with type of wound), 4)

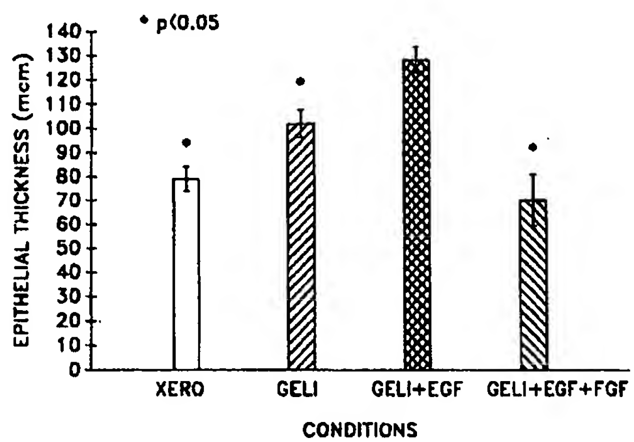

Figure 3. Epithelial thickness (micrometers) of closed central interstices of controls, and grafts receiving epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) plus EGF. Grafts with bFGF alone had no measureable closed interstices.

not effecting the wound in its own way, 5) consistent release of the factor, 6) being non-toxic and 7) ability to be easily handled. In this experiment we have used a polyacrylamide gel (Geliperm), as it meets the requirements for an excellent delivery vehicle on the athymic mouse. Its allows gradual release of both EGF and bFGF into the wound bed. Approximately 90% of the EGF is delivered to the wound within 24 hours, while bFGF is delivered somewhat slower. At 48 hours, the time between dressing changes, one third of the bFGF is estimated to be released. (Data supplied by Amgen Incorporated) Thus approximately 100 ng/cm2 is delivered to the wound each dressing change, which is the desired dose. Geliperm may not be as effective in the clinical setting, as it may stay more moist than desired. The problem of attaching this dressing to the wound is also being evaluated.

The effect of EGF on epithelization in this model was substantial. Time to closure of those wounds treated with EGF was significantly shortened. The dose of 10 mcg/cm2 was similar to doses used in other studies (Laato et al., 1985, Buckley et al., 1985, Brown et al., 1986, Brown et al., 1989, Thorton et al., 1981, and Chvapil et al., 1988). Franklin used considerably more EGF (Franklin and Lynch, 1979). At these doses no obvious toxicity was seen, but no specific testing was done to determine this.

Contrary to the EGF results was the significant deleterious effect of bFGF to epithelialization of the interstices in this initial study. The interstices stayed open much longer, and in some mice the bridges of meshed skin appeared to dissolve. This was accompanied by a beefy granulation bed over the entire wound.

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Histologies confirmed the absence of a regenerated epithelium, and the formation of new, vascularized tissue at the interstices. The dose of bFGF was 300 ng/cm2, but as previously mentioned, the amount delivered to the wound was approximately 1/3 of this. This dose is considerably less than used by some investigators (Lazarou et al., 1989) and similar to others (McGee et al., 1988 and Eriksson et al., 1989). These preliminary findings are important in that bFGF may not be an appropriate growth factor to incorporate into clinical studies evaluating partial thickness, acute wounds. Further work with different titrations of EGF and bFGF are needed to fully determine their potential efficacy.

This model was designed to examine the effects of various agents on epithelialization in wound healing. It can be used to study the delivery of multiple agents, including growth factors, attachment peptides, antimicrobial agents and other wound altering substances. It addresses the epithelialization of human skin across a vascular bed from the athymic mouse. Human keratinocytes migrate across the open interstices as the only form of closure. But the bed itself is important, and many of the peptide growth factors tested will have great effects on the vascularization, fibroplasia, collagen formation and organization of the advancing keratinocytes.

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REGRANEX*GEL 0.01% (becaplermin)

Prescribing Information

DESCRIPTION
REGRANEX® Gel contains becaplermin, a recombinant human platelet-derived growth factor (rhPDGF-BB) for topical administration. Becaplermin is produced by recombinant DNA technology by insertion of the gene for the B chain of platelet-derived growth factor (PDGF) into the yeast, *Saccharomyces cerevisiae*. Becaplermin has a molecular weight of approximately 25 KD and is a homodimer composed of two identical polypeptide chains that are bound together by disulfide bonds. Becaplermin Concentrate is produced by Chiron Corp. and supplied to OMJ Pharmaceuticals under a shared manufacturing arrangement. REGRANEX Gel is a non-sterile, low bioburden, preserved, sodium carboxymethylcellulose-based (CMC) topical gel, containing the active ingredient becaplermin and the following inactive ingredients: sodium chloride, sodium acetate trihydrate, glacial acetic acid, water for injection, and methylparaben, propylparaben, and m-cresol as preservatives and l-lysine hydrochloride as a stabilizer. Each gram of REGRANEX Gel contains 100 µg of becaplermin.

CLINICAL PHARMACOLOGY
REGRANEX has biological activity similar to that of endogenous platelet-derived growth factor, which includes promoting the chemotactic recruitment and proliferation of cells involved in wound repair and enhancing the formation of granulation tissue.

Pharmacokinetics
Ten patients with Stage III or IV (as defined in the International Association of Enterostomal Therapy (IAET) guide to chronic wound staging, *J. Enterostomal Ther* 15:4, 1988 and *Decubitis* 2:24, 1989) lower extremity diabetic ulcers received topical applications of becaplermin gel 0.01% at a dose range of 0.32-2.95 µg/kg (7µg/cm²) daily for 14 days. Six patients had non-quantifiable PDGF levels at baseline and throughout the study, two patients had PDGF levels at baseline which did not increase substantially, and two patients had PDGF levels that increased sporadically above their baseline values during the 14 day study period.

Systemic bioavailability of becaplermin was less than 3% in rats with full thickness wounds receiving single or multiple (5 days) topical applications of 127 µg/kg (20.1 µg/cm2 of wound area) of becaplermin gel.

Clinical Studies
The effects of REGRANEX Gel on the incidence of and time to complete healing in lower extremity diabetic ulcers were assessed in four randomized controlled studies. Of 922 patients studied, 478 received either REGRANEX Gel 0.003% or 0.01%. All study participants had lower extremity diabetic neuropathic ulcers that extended into the subcutaneous tissue or beyond (Stages III and IV of the IAET guide to chronic wound staging). Ninety-three percent of the patients enrolled in these four trials had foot ulcers. The remaining 7% of the patients had ankle or leg ulcers. The diabetic ulcers were of at least 8 weeks duration and had an adequate blood supply (defined as $TcpO^2 > 30$ mm Hg). In the four trials, ninety-five percent of the ulcers measured in area up to 10 cm², and the median ulcer size at baseline ranged from 1.4 cm² to 3.5 cm². All treatment groups received a program of good ulcer care consisting of initial complete sharp debridement, a non-weight-bearing regimen, systemic treatment for wound-related infection if present, moist saline dressings changed twice a day, and additional debridement as necessary. REGRANEX Gel 0.003% or 0.01% or placebo gel was applied once a day and covered with a saline moistened dressing. After approximately 12 hours, the gel was gently rinsed off and a saline moistened dressing was then applied for the remainder of the day. Patients were treated until complete healing, or for a period of up to 20 weeks. Patients were considered a treatment failure if their ulcer did not show an approximately 30% reduction in initial ulcer area after eight to ten weeks of REGRANEX Gel therapy.

The primary endpoint, incidence of complete ulcer closure within 20 weeks, for all treatment arms is shown in Figure 1. In each study, REGRANEX Gel in conjunction with good ulcer care was compared to placebo gel plus good ulcer care or good ulcer care alone.

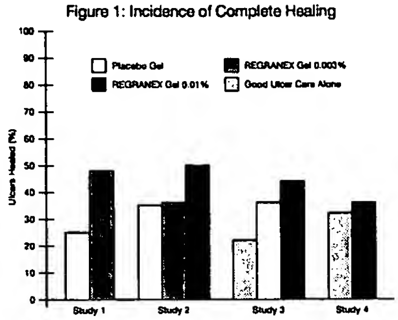
In Study 1, a multicenter, double-blind, placebo controlled trial of 118 patients, the incidence of complete ulcer closure for REGRANEX Gel 0.003% (n=61) was 48% versus 25% for placebo gel (n=57; p=0.02, logistic regression analysis).

In Study 2, a multicenter, double-blind, placebo controlled trial of 382 patients, the incidence of complete ulcer closure for REGRANEX Gel 0.01% (n=123), was 50% versus 36% for REGRANEX Gel 0.003% (n=132), and 35% for placebo gel (n=127). Only REGRANEX Gel 0.01% was significantly different from placebo gel (p=0.01, logistic regression analysis).

The primary goal of Study 3, a multicenter controlled trial of 172 patients, was to assess the safety of vehicle gel (placebo; n=70) compared to good ulcer care alone (n=68). The study included a small (n=34) REGRANEX Gel 0.01% arm. Incidences of complete ulcer closure were 44% for REGRANEX Gel, 36% for placebo gel and 22% for good ulcer care alone.

In Study 4, a multicenter, evaluator-blind, controlled trial of 250 patients, the incidences of complete ulcer closure in the REGRANEX Gel 0.01% arm (n=128) (36%) and good ulcer care alone (n=122) (32%) were not statistically different.

Figure 1: Incidence of Complete Healing



In general, where REGRANEX Gel was associated with higher incidences of complete ulcer closure, differences in the incidence first became apparent after approximately 10 weeks and increased with continued treatment (Table 1).

Table 1: Life Table Estimates of the Incidence (%) of Complete Healing Over Time for Study 2

	REGRANEX Gel 0.01% (%)	Placebo Gel (%)
Week 2	1	0
Week 4	6	2
Week 6	9	6
Week 8	16	14
Week 10	23	18
Week 12	34	25
Week 14	37	28
Week 16	43	33
Week 18	46	34
Week 20	50	37

In a 3-month follow-up period where no standardized regimen of preventative care was utilized, the incidence of ulcer recurrence was approximately 30% in all treatment groups, demonstrating that the durability of ulcer closure was comparable in all treatment groups.

The efficacy of REGRANEX Gel for the treatment of non-diabetic ulcers is under evaluation.

INDICATIONS AND USAGE
REGRANEX Gel is indicated for the treatment of lower extremity diabetic neuropathic ulcers that extend into the subcutaneous tissue or beyond and have an adequate blood supply. When used as an adjunct to, and not a substitute for, good ulcer care practices including initial sharp debridement, pressure relief and infection control, REGRANEX Gel increases the incidence of complete healing of diabetic ulcers.

The efficacy of REGRANEX Gel for the treatment of diabetic neuropathic ulcers that do not extend through the dermis into subcutaneous tissue (Stage I or II, IAET staging classification) or ischemic diabetic ulcers has not been evaluated.

CONTRAINDICATIONS
REGRANEX Gel is contraindicated in patients with:
- known hypersensitivity to any component of this product (e.g., parabens);
- known neoplasm(s) at the site(s) of application.

WARNINGS
REGRANEX (becaplermin) Gel is a non-sterile, low bioburden preserved product. Therefore, it should not be used in wounds that close by primary intention.

PRECAUTIONS
For external use only.

If application site reactions occur, the possibility of sensitization or irritation caused by parabens or m-cresol should be considered.

The effects of becaplermin on exposed joints, tendons, ligaments, and bone have not been established in humans. In pre-clinical studies, rats injected at the metatarsals with 3 or 10 µg/site (approximately 60 or 200 µg/kg) of becaplermin every other day for 13 days displayed histological

changes indicative of accelerated bone remodeling consisting of periosteal hyperplasia and subperiosteal bone resorption and exostosis. The soft tissue adjacent to the injection site had fibroplasia with accompanying mononuclear cell infiltration reflective of the ability of PDGF to stimulate connective tissue growth.

Information for Patients
Patients should be advised that:

- hands should be washed thoroughly before applying REGRANEX Gel;
- the tip of the tube should not come into contact with the ulcer or any other surface; the tube should be recapped tightly after each use;
- a cotton swab, tongue depressor, or other application aid should be used to apply REGRANEX Gel;
- REGRANEX Gel should only be applied once a day in a carefully measured quantity (see Dosage and Administration section). The measured quantity of gel should be spread evenly over the ulcerated area to yield a thin continuous layer of approximately 1/16 of an inch thickness. The measured length of the gel to be squeezed from the tube should be adjusted according to the size of the ulcer. The amount of REGRANEX Gel to be applied daily should be recalculated at weekly or biweekly intervals by the physician or wound care giver;

Step-by-step instructions for application of REGRANEX Gel are as follows:

- Squeeze the calculated length of gel on to a clean, firm, non-absorbable surface, e.g., wax paper.
- With a clean cotton swab, tongue depressor, or similar application aid, spread the measured REGRANEX Gel over the ulcer surface to obtain an even layer.
- Cover with a saline moistened gauze dressing.

- after approximately 12 hours, the ulcer should be gently rinsed with saline or water to remove residual gel and covered with a saline-moistened gauze dressing (without REGRANEX Gel);
- it is important to use REGRANEX Gel together with a good ulcer care program, including a strict non-weight-bearing program;
- excess application of REGRANEX Gel has not been shown to be beneficial;
- REGRANEX Gel should be stored in the refrigerator. Do not freeze REGRANEX Gel;
- REGRANEX Gel should not be used after the expiration date on the bottom, crimped end of the tube.

Drug Interactions
It is not known if REGRANEX Gel interacts with other topical medications applied to the ulcer site. The use of REGRANEX Gel with other topical drugs has not been studied.

Carcinogenesis, Mutagenesis, Impairment of Fertility
Becaplermin was not genotoxic in a battery of in vitro assays, (including those for bacterial and mammalian cell point mutation, chromosomal aberration, and DNA damage/repair). Becaplermin was also not mutagenic in an *in vivo* assay for the induction of micronuclei in mouse bone marrow cells.

Carcinogenesis and reproductive toxicity studies have not been conducted with REGRANEX Gel.

Pregnancy: Category C
Animal reproduction studies have not been conducted with REGRANEX Gel. It is also not known whether REGRANEX Gel can cause fetal harm when administered to a pregnant woman or can affect reproductive capacity. REGRANEX Gel should be given to pregnant women only if clearly needed.

Nursing Mothers
It is not known whether becaplermin is excreted in human milk. Because many drugs are secreted in human milk, caution should be exercised when REGRANEX Gel is administered to nursing women.

Pediatric Use
Safety and effectiveness of REGRANEX Gel in pediatric patients below the age of 16 years have not been established.

ADVERSE REACTIONS

Patients receiving REGRANEX Gel, placebo gel, and good ulcer care alone had a similar incidence of ulcer-related adverse events such as infection, cellulitis, or osteomyelitis. However, erythematous rashes occurred in 2% of patients treated with REGRANEX Gel and placebo, and none in patients receiving good ulcer care alone. The incidence of cardiovascular, respiratory, musculoskeletal and central and peripheral nervous system disorders was not different across all treatment groups. Mortality rates were also similar across all treatment groups. Patients treated with REGRANEX Gel did not develop neutralizing antibodies against becaplermin.

DOSAGE AND ADMINISTRATION

The amount of REGRANEX Gel to be applied will vary depending upon the size of the ulcer area. To calculate the length of gel to apply to the ulcer, measure the greatest length of the ulcer by the greatest width of the ulcer in either inches or centimeters. To calculate the length of gel in inches, use the formula shown below in Table 2, and to calculate the length of gel in centimeters, use the formula shown below in Table 3.

Table 2: Formula to Calculate Length of Gel in Inches
to be Applied Daily

INCHES

Tube Size	Formula
15 or 7.5g tube	length X width X 0.6
2g tube	length X width X 1.3

Using the calculation, each square inch of ulcer surface will require approximately ⅗ inch length of gel squeezed from a 15g or 7.5g tube, or approximately 1⅓ inch length of the gel from a 2g tube. For example, if the ulcer measures 1 inch by 2 inches, then a 1¼ inch length of gel should be used for 15g or 7.5g tubes (1 X 2 X 0.6 = 1¼) and 2¾ inch gel length should be used for 2g tube (1 X 2 X 1.3 = 2¾).

Table 3: Formula to Calculate Length of Gel in Centimeters
to be Applied Daily

CENTIMETERS

Tube Size	Formula
15 or 7.5g tube	length X width ÷ 4
2g tube	length X width ÷ 2

Using the calculations for ulcer size in centimeters, each square centimeter of ulcer surface will require approximately a 0.25 centimeter length of gel squeezed from a 15g or 7.5g tube, or approximately a 0.5 centimeter length of gel from a 2g tube. For example, if the ulcer measures 4 cm by 2 cm, then a 2 centimeter length of gel should be used for 15g or 7.5g tube [(4 X 2) ÷ 4 = 2] and a 4 centimeter length of gel should be used for 2g tube [(4 X 2) ÷ 2 = 4].

The amount of REGRANEX Gel to be applied should be recalculated by the physician or wound care giver at weekly or biweekly intervals depending on the rate of change in ulcer area. The weight of REGRANEX Gel from 7.5g and 15g tubes is 0.65g per inch length and 0.25g per centimeter length.

To apply REGRANEX Gel, the calculated length of gel should be squeezed on to a clean measuring surface, e.g., wax paper. The measured REGRANEX Gel is transferred from the clean measuring surface using an application aid and then spread over the entire ulcer area to yield a thin continuous layer of approximately 1/16 of an inch thickness. The site(s) of application should then be covered by a saline moistened dressing and left in place for approximately 12 hours. The dressing should then be removed and the ulcer rinsed with saline or water to remove residual gel and covered again with a second moist dressing (without REGRANEX Gel) for the remainder of the day. REGRANEX Gel should be applied once daily to the ulcer until complete healing has occurred. If the ulcer does not decrease in size by approximately 30% after 10 weeks of treatment or complete healing has not occurred in 20 weeks, continued treatment with REGRANEX Gel should be reassessed. The step-by-step instructions for applying REGRANEX Gel for home administration are described under "Information for Patients".

HOW SUPPLIED

REGRANEX (becaplermin) Gel, supplied as a clear, colorless to straw-colored preserved gel containing 100μg of becaplermin per gram of gel, is available in multi-use tubes in the following sizes:

2g tubes	NDC 0045-0810-02
7.5g tubes	NDC 0045-0810-07
15g tubes	NDC 0045-0810-15

REGRANEX Gel is for external use only.

Storage
Store refrigerated, 2-8°C (36-46°F). DO NOT FREEZE. DO NOT USE THE GEL AFTER THE EXPIRATION DATE AT THE BOTTOM OF THE TUBE.

U.S. Patent #5,457,093

ORTHO-McNEIL

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